

Patent Application  
Attorney Docket No. 3153.00050/PC10299A

**AMENDED VERSION**

**IN THE SPECIFICATION:**

**Beginning on page 3, line 25 and ending on page 4, line 4, please delete and insert therefor:**

C In a preferred embodiment, the culture is inactivated by adding formalin (about 0.5% v/v final concentration). In another preferred embodiment, antigens of the invention are obtained from the supernatant or filtrate of an *E. rhusiopathiae* culture. A culture supernatant or filtrate, in a preferred embodiment, is concentrated about 10-fold and aluminum hydroxide gel (preferably REHYDRAGEL™) is added to the concentrated supernatant or filtrate at a final concentration of about 30% v/v to stabilize the antigen. In another preferred embodiment, a vaccine composition is formulated comprising the antigen and an adjuvant with the adjuvant comprising, for example, about 25% v/v of the vaccine composition. In another preferred embodiment, thimerosal (about 0.01% v/v final concentration) with EDTA (about 0.07% v/v final concentration) are added to the antigens as preservative. A preferred adjuvant, herein referred to as "No.1 Adjuvant", comprises about 2% v/v lecithin, about 18% v/v mineral oil, and about 8% v/v surfactant (e.g., about 5.6% v/v TWEEN 80™ and about 2.4% v/v SPAN 80™), with the remaining volume being a saline solution (e.g., Dulbecco PBS). This adjuvant is described in U.S. Patent Application Serial No. 60/117,705, filed January 29, 1999, entitled "Adjuvants for Use in Vaccines", which is incorporated herein by reference.

**Beginning on page 7, line 19 and ending on page 8, line 3, please delete and insert therefor:**

C An antigen of the invention may be used in a vaccine composition to immunize an animal. In one embodiment, the vaccine composition contains an antigen of the invention and an adjuvant. In a preferred embodiment, an adjuvant useful for a vaccine composition of the invention comprises a lecithin, an oil, and a surfactant. A vaccine composition formulated with a preferred adjuvant contains a lecithin at from about 0.25% to about 12.5% v/v, more preferably from about 0.5% to about 5%, and most preferably from about 0.5% to about 1.25% v/v, an oil at from about 1% to about 23% v/v, more preferably from about 3.5% to about 10% and most preferably about 4.5%, and an amphiphilic surfactant at from about 1.5% to about 6% v/v, more preferably from about 1.5% to about 4% and most preferably about 2% v/v. Preferably the adjuvant has 2 amphiphilic surfactants, for example TWEEN™ and SPAN™ surfactants, of which one predominantly in the aqueous phase (e.g., TWEEN 80™) of the vaccine composition and one in the oil phase (e.g., SPAN 80™). Preferably, when TWEEN 80™ and SPAN 80™ are used as

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C<sup>2</sup> surfactants, the concentration of TWEEN 80™ is about 1½ to about 3 times as high as the concentration of SPAN 80™, preferably about 2 times. A preferred adjuvant contains an aqueous carrier solution, for example, phosphate-buffered saline (PBS) ( .g., Dulbecco PBS). A lecithin and an oil suitable for an adjuvant for the vaccine compositions is a mixture of lecithin in DRAKEOL™ 5 Lt Mineral Oil. Lecithin may be obtained from Central Soya, Fort Wayne, Indiana. See also U.S. Patent No. 5,084,269, which discusses adjuvant compositions. TWEEN™ and SPAN™ surfactants may be obtained from Van Waters and Rogers, Omaha, Nebraska.

Beginning on page 10, line 27 and ending on line 31, please delete and insert therefor:

C<sup>3</sup> *E. rhusiopathiae* strain CN 3342 is cultured in medium containing Difco Proteose Peptone at a concentration of 2.75%, Difco Yeast Extract (0.55%), TWEEN 80™ (0.2%), K<sub>2</sub>HPO<sub>4</sub> (0.217%) and KH<sub>2</sub>PO<sub>4</sub> (0.061%) in deionized water. The pH of the medium is adjusted to 7.2 with 5N NaOH. The medium is steam sterilized at a minimum of 122° C for 30 to 90 minutes. After autoclaving, sterile 50% dextrose solution is added to a final concentration of 3% w/v.

Beginning on page 12, line 18 and ending on line 27, please delete and insert therefor:

C<sup>4</sup> The adjuvant used was No.1 Adjuvant. 1000 mL of No.1 Adjuvant were made from 200 mL filter sterilized lecithin-oil solution (10% lecithin in DRAKEOL™ mineral oil), autoclaved TWEEN 80™ (56 mL) and SPAN 80™ (24 mL), and phosphate buffered saline (Dulbecco PPPBS) (720 mL). The lecithin-oil solution and SPAN 80™ were combined and mixed in a sterile tank for at least 1 hour at room temperature until emulsification was complete. The saline and TWEEN 80™ were combined and mixed in a sterile tank for at least 1 hour at room temperature. The oil mixture was emulsified with the aqueous mixture using a Ross emulsifier. Emulsification was continued by recirculation until all of the adjuvant was added into the saline. The emulsion was then passed twice through a Gaulin press at room temperature. The adjuvant was stored at 2 to 8° C.

Beginning on page 15, line 7 and ending on line 30, please delete and insert therefor:

C<sup>5</sup> Sows were bled 0 to 10 days prior to farrowing to determine their *E. rhusiopathiae* antibody titers. Piglets were randomized based on sows' serological titers and farrowing dates. Fifty eight (58) piglets derived from these sows/gilts were bled and vaccinated at approximately 3 weeks of age with one of the two experimental *E. rhusiopathiae* vaccines or the placebo (groups listed in Table 1). At approximately 4 weeks of age the piglets were weaned. At approximately 6